On Golgi's Internal Apparatus in spontaneously absorbing Tumour Cells.[1]

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With Plates 19 and 20.

Introductory.

The results obtained by the study of the Golgi apparatus of growing tumours of the mouse, rat, and guinea-pig were set forth in a previous paper (**7**) in which also the literature of the subject was summarized and discussed. The apparatus was found to be constantly present in the healthy cells of all tumours examined, in most of which it appeared with certain characteristic features. These were maintained through the successive regrafting of the same growths even when the general histological picture of some of them had somewhat changed and the tumour cells and their apparatus had become hypertrophic or had undergone partial degeneration.

It is now proposed to describe the behaviour of the apparatus during the spontaneous absorption of some of the same tumours, a point which could not be properly dealt with in the previous work. Such an investigation has not, as yet, been carried out, though it is important because it gives the opportunity for studying the modifications of the apparatus in cells of either epithelial or connective-tissue origin, undergoing regressive changes after a period of active proliferation, a phenomenon obtained with difficulty under different experimental conditions.

As shown in another paper (**6**), spontaneously absorbing

[1] Part of the expenditure incurred for carrying out the present research was defrayed by a grant from the Royal Society. The material was obtained through the kindness of Dr. J. A. Murray, Director of the Laboratories of the Imperial Cancer Research Fund.

tumours of small laboratory animals are at first invaded by elements of the lymphocytic type and then by connective-tissue cells, the process generally ending in the complete disappearance of the tumour cells and the production of a scar structurally similar to that seen during the healing of certain inflammatory lesions of the subcutaneous connective tissue. When studying the apparatus of absorbing tumour cells, that of some of the elements taking part in the production of the scar was observed and will be also briefly described.

Material and Methods.

The following malignant new growths in various phases of spontaneous absorption were examined:

Mouse.

Jensen. Alveolar carcinoma.
Twort. Alveolar carcinoma.
27. Papillary cystic adeno-carcinoma.
91. Haemorrhagic and cystic alveolar carcinoma.
113. Slightly haemorrhagic alveolar carcinoma.
155. Fissure forming adeno-carcinoma.
206. Alveolar carcinoma.
630. Squamous cell carcinoma.
37 S. Spindle cell sarcoma.

Rat.

Rat 9. Adeno-carcinoma with dense hyaline stroma.

All tumours were investigated by the cobalt nitrate method with the precautions suggested in the previous work. A certain number of tumours were likewise investigated by the modification of Veratti's potassium antimoniate method previously described. But this time the results obtained were not so satisfactory as when investigating the apparatus of growing tumours. This was probably due to the fact that absorbing new growths often contain only a small number of healthy cells and a great deal of detritus which becomes so intensely impregnated by the potassium antimoniate method as to render

extremely difficult the interpretation of the histological pictures. It is necessary to add here that a description of Veratti's original method was published by Barinetti in a paper which had been previously overlooked (**1**).

No conclusive results were reached by the investigation of the Rat 9 carcinoma either by the cobalt nitrate or by the potassium antimoniate methods in spite of repeated attempts made in different stages of the absorption process. Successful impregnations were obtained only when most of the tumour cells were in a healthy condition, and it is therefore proposed not to include this tumour in the following description. However, the fact was worth mentioning because it has a parallel in a previous observation regarding a transplantable liposarcoma of the guinea-pig. In that case satisfactory results were obtained by Golgi's arsenious acid method, but not by others. The two observations taken together seem to indicate that biological conditions, through which tissues may be passing, have sometimes a decisive influence on the reaction to which the silver impregnation is due. Ernst's (**10**) recent investigations on certain phenomena of adsorption are in favour of this supposition.

The Apparatus of Spontaneously Absorbing Carcinomata and Sarcomata of the Mouse.

Jensen.—As previously described, the apparatus of the healthy cells of this tumour has a perinuclear or juxta-nuclear position, and generally consists of minor parts ring- or loop-like in shape. This is also seen in the small group of unaltered cells shown on one side of Pl. 19, fig. 1, which was drawn from a zone of transition between a surviving nodule and an absorption area. In most of the degenerating tumour cells the apparatus is recognized because of its characteristic aspect, the ring- and loop-like shapes being scattered in the cytoplasm instead of being collected in one juxta-nuclear formation. Only in a relatively small number of cells an irregular fragmentation of the apparatus is observed, though this phenomenon becomes more and more apparent and lastly predominates

when and where the absorption process is most advanced. In such places, however, the degeneration of the tumour cells has gone so far that they are hardly recognizable.

In transitional zones like that shown in Pl. 19, fig. 1, multinucleated protoplasmic masses are now and then observed, apparently due to the conglutination of a variable number of degenerating tumour cells. Next to almost each of the nuclei included in these pseudo-giant cells, typical remnants of the apparatus are frequently seen with arrangements similar to that described in connexion with a polymorphous cell sarcoma of the mouse (see Pl. xxi, fig. 29, of the previous work, 7). A fusion of the apparatus of the single cells into a common and centrally situated formation, as observed under different conditions, was not met with in specimens from the Jensen carcinoma.

When the absorption process is nearing its end and the connective-tissue proliferation leading to the formation of the scar is very much advanced, the number of the carcinomatous cells is very small, and these are recognized with difficulty, particularly by the method used in this investigation. Nevertheless elements are now and then found which can be safely considered as surviving tumour cells. Their identification is chiefly based on the size and aspect of their nuclei and apparatus. As shown by Pl. 19, fig. 2, this has a much more robust appearance than that of the other cells, most of which are large wandering cells and fibroblasts mixed with a few leucocytes. In some of the tumour cells the apparatus still possesses the characteristic aspect to which reference has already been made ; in others it is uncommonly large and looks like a somewhat granular and disintegrating juxta-nuclear structure. The apparatus of the connective-tissue cells is much smaller, irregular in shape and structure, and often stretched in various directions.

T w o r t.—The healthy cells of this tumour are provided with an apparatus in the main smaller and more distinctly reticular than that of Jensen's carcinoma. The changes observed during absorption are similar to those above described,

though a simple disintegration of the apparatus into a structureless material was the prevalent feature of many specimens. A transformation of the apparatus into a large juxta-nuclear formation was sometimes observed (Pl. 19, fig. 3, *tu.*), but not the fusion of various elements into pseudo-giant cells as in the case of the Jensen tumour.

In advanced stages of absorption of the Twort carcinoma accumulations of large macrophages were found. Most of them contained only formless débris of argentophile material, but some possessed a small and irregularly shaped apparatus (Pl. 19, fig. 3, *m.*) situated close to the nucleus and on the whole similar to that of the connective-tissue elements above mentioned.

Tumour 27.—The apparatus of the healthy cells of this adeno-carcinoma generally consists of short rods collected in a bunch on that side of the nucleus which is turned towards an existing or virtual glandular lumen. Only in some groups of cells it appears formed of reticular portions irregular in size, shape, and distribution. In absorbing tumours of the same strain the typical rod-like aspect survives only in a rather small number of cells. Most of them either show the picture occasionally observed in growing tumours or convey the impression that the rods forming the apparatus have swollen into elliptical or roundish shapes within which a minute light space can be detected. It has been impossible to decide whether these spaces are really empty or contain a material which does not take the silver. Pl. 19, fig. 4, is a good instance of this condition which, in the specimens investigated, extended to wide areas, easily distinguished from the unaltered tumour portions by the pale colour of the nuclei. These showed in addition a strong tendency to fuse into agglomerations in which the boundaries between cell and cell could hardly be made out even by very high magnifications.

These observations were at first found a little surprising. Other absorbing tumours of the same strain were therefore carefully investigated, but with results which did not essentially differ from those already obtained. The number

of cells provided with an apparatus of the characteristic type described varied considerably from tumour to tumour and from place to place of the same tumour : but whenever the absorption process had manifested itself, the apparatus showed in varying degrees the changes shown in Pl. 19, fig. 4.

It is interesting to note that the same fact was even more clearly observed in dividing tumour cells. Mitotically dividing cells frequently occur in spontaneously absorbing tumours even when these are much reduced in size and nearing complete disappearance. One of such cells is shown in a part of Pl. 19, fig. 4, and another in the centre of Pl. 20, fig. 5. In both cases the fragments of the apparatus (dictyosomes) to be subdivided between the daughter cells no longer have the aspect generally observed in growing tumours of small solid clumps of argentophile material, but appear as irregularly rounded or elliptical shapes with a light central portion, as in most of the absorbing cells at rest. Pl. 20, fig. 5, was drawn from a specimen of a tumour in a very advanced phase of absorption, and the apparatus of some cells show signs of the approaching complete disintegration.

Tumours 91 and 206.—The apparatus of the healthy cells of these two growths has a fine reticular structure, more delicate in carcinoma 206 than in carcinoma 91. During absorption the characteristic aspect of the apparatus is still recognized for a time in the cells of both of them. In the case of tumour 91, however, the apparatus is soon reduced to a granular material with loss of the previous reticular arrangement. This is well shown in Pl. 20, fig. 6, which was drawn from a place where the various phases of this degenerative process could be seen one next to the other. In other areas of the same absorbing tumour, appearances almost identical with that exhibited by the central portion of Pl. xxii, fig. 32, of the previous work were sometimes noticed. In such instances the similarity between the disintegrative phenomena affecting the apparatus of both the tumours now considered was rather striking, though in carcinoma 206 the gradual fragmentation of the previous fine network was plainer. As shown by Pl. 20, fig. 7, a reticula

appearance still survived in spite of the breaking up of the whole structure into small portions and threads, only now and then united by thinner filaments.

Tumours 113 and 155.—The apparatus of the healthy cells of these two carcinomata is very small and therefore less suitable for observations of the kind considered in the present paper. In the cells of areas undergoing absorption the apparatus occurred, in both tumours, in the form of short rods or small clumps closely arranged, one next to the other, on one pole of the nuclei, but no structural details could be made out in the intensely impregnated fragments. In more advanced phases of absorption these fragments were still smaller and frequently indistinguishable from formless débris.

In conditions of this sort elements provided with a small and well impregnated apparatus were often seen, but accurate observations, particularly of serial sections, led to the conclusion that such elements were connective-tissue cells similar to those shown in part of Pl. xxii, fig. 32, of the previous work and in Pl. 19, fig. 2, of the present one.

Tumour 630.—The regressive changes exhibited during absorption by this squamous cell carcinoma are identical with those previously observed in keratinizing areas of the same tumour and need no further description. When absorption is very advanced the tumours consist of small nodules of a more or less completely keratinized material surrounded by a shell of proliferated connective tissue in which a considerable number of multinucleated giant cells are found. As shown by Pl. 20, fig. 8, some of them are provided with remnants of a centrally situated and reticular apparatus, some only contain a finely granular material, the granules being sometimes arranged in such a way as to convey the impression that they also have arisen from the fragmentation of a perhaps formerly reticular apparatus. The presence of many giant cells in the above-mentioned situation is probably due to the fact that in the absorption phase in which they were noticed, nothing survived of the old tumour but a hard substance in many respects similar to a foreign body. The fragmentation of their apparatus may

be attributed to the process of involution through which also the proliferated connective-tissue cells presumably pass before disappearing with the remains of the tumour.

Together with the giant cells, either in the same situations or between the keratinized nodules, elements of the large wandering cell type were frequently observed. They were provided with a small either reticular or compact apparatus similar to that of elements of the same kind previously mentioned and described also by Verson (17), though in different pathological conditions.

37 S.—In the absorption areas of this sarcoma of the mouse the apparatus undergoes a simple process of granular disintegration, frequently manifesting itself at a period in which the outward aspect and structure of the tumour cells is otherwise almost unaltered. Apart from the size of the cells, the phenomenon is like that observed in the foreign body giant cells described in connexion with Pl. 20, fig. 8. As easily deduced from a comparison between Pl. 20, figs. 8 and 9, in both cases the apparatus soon becomes transformed into an accumulation of small argentophile granules, the arrangement of which now and then reminds one of a pre-existing reticular structure. This observation is not without interest, because the cells exhibiting the changes described have in common a connective-tissue origin. Pl. 20, fig. 9, was drawn from the peripheral portion of a nodule, the central cells of which still appeared in a healthy condition and possessed an apparatus identical with that shown in Pl. xvi, fig. 19, of the previous work. Only a few of the peripherally situated cells were provided with an intact apparatus, though remains of it could be recognized even in elements showing many signs of degeneration, such as loss of a great part of the nuclear chromatin, liquefaction, and fusion of the cell-bodies.

General Considerations.

One of the principal facts resulting from the present investigation is the relative resistance of the apparatus to altered biological conditions even when these lead to a complete

degeneration of the cells concerned. The moment, in which the disintegration of the apparatus begins, appears to vary within wide limits and for causes which are beyond our present means of observation. However, changes of the apparatus are on the whole noticed when the external aspect and structure of the cells seem otherwise unaltered. After this initial phase a period follows during which portions and fragments of the apparatus continue to be recognized until the extreme degree of cell degeneration is reached. This is in agreement with previous observations on growing transplantable tumours (**7**) and with the results obtained by other authors in different fields of investigation. It corroborates the suggestion resulting from Bowen's recent work (**4, 5**) that the apparatus plays perhaps an important rôle in the economy of the cell, though we must confess with him our ignorance as to the exact nature of this rôle.

A second point deserving brief discussion is the different behaviour of the apparatus during the absorption of different tumours. In some of them, as in certain connective-tissue cells, it soon becomes transformed into a granular material which, though persisting during cell degeneration, no longer possesses any definite structure; in others this terminal disintegration is reached through stages during which it either swells into peculiar shapes or breaks into fragments and pieces which are for a time endowed with certain distinctive features. In certain cases it even passes through a sort of hypertrophic condition which, in the material examined, could not be more closely studied. These phenomena are probably influenced, if not determined, by the different structure of the apparatus in the healthy cells of the tumours investigated, and confirm the opinion previously expressed as to the existence of a well-defined relation between the apparent structural type and the mode of being of the apparatus in the living cells.

The disintegration of the apparatus during the spontaneous absorption of certain tumours has a parallel in the observations made by other authors and myself under different pathological or physiological conditions. For instance, the breaking up of

the apparatus in certain growths (Jensen's carcinoma, tumours 206 and 91) has a striking similarity with Penfield's retispersion (**15, 16**) and with alterations of the same kind observed in degenerating nerve-cells by Battistessa (**2, 3**), Marcora (**14**), Da Fano (**8**), and others. Some of the changes exhibited by the apparatus in tumour 27 have an almost surprising resemblance with the modifications exhibited by the apparatus in germ-cells as described by Gatenby (**11**) and his collaborators Woodger (**12**) and Ludford (**13**), and more recently by Bowen (**5**). The same applies to the changes noticed by Da Fano (**9**) during the involution of the mammary gland at the end of lactation. The phenomena described by the above-mentioned authors and in the present paper were noticed in tissues so different that no detailed comparison is possible. However, they were worth brief mention because they seem to indicate that the fragmentation of the apparatus, whether under the influence of physiological or pathological stimuli, is up to a point determined by the as yet obscure part taken by the apparatus in various cell activities.

Summary.

In continuation of previous work the behaviour of Golgi's apparatus during spontaneous absorption of transplantable tumours of the mouse is described. In some of them the apparatus soon becomes transformed into a granular almost structureless material; in others this terminal phase is reached through stages which are probably determined by the characteristic structure exhibited by the apparatus in the healthy cells of the corresponding tumours. The modifications of the apparatus during such stages have a striking resemblance with the changes observed in different tissues under various physiological conditions. The connective-tissue cells, which invade the tumours as they are absorbed, are provided with a small, irregular, and sometimes reticular apparatus which is identical with that described by other authors in similar elements though in different pathological processes. In some of these elements

the apparatus undergoes changes not altogether different from those observed in absorbing tumour cells. In general, the fragmentation of the apparatus begins when other cell constituents are still apparently unaltered, but its fragments seem to possess a great power of resistance to degenerative conditions.

London,
March 1923.

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DESCRIPTION OF PLATES 19 and 20.

All figures have been drawn by means of the camera lucida at the magnification indicated for each of them, and are from spontaneously absorbing tumours treated by the cobalt nitrate method for the demonstration of Golgi's internal apparatus. They are fully described in the text.

Reference Letters.

d.tu., degenerating tumour cell; *f.*, fibroblast; *g.c.*, giant cell; *ps.g.c.*, pseudo-giant cell; *k.*, area of keratinization; *m.*, macrophage; *plc.*, polymorphonuclear leucocyte; *tu.*, tumour cell; *w.c.*, wandering cell.

Fig. 1.—Jensen's carcinoma (387 A). Moderately progressed absorption. (×1160.)

Fig. 2.—Jensen's carcinoma (399 B). Advanced absorption; surviving tumour elements amongst proliferated connective-tissue cells. (×1620.)

Fig. 3.—Twort's carcinoma (123 A) at the end of the absorption process. One surviving tumour cell; group of macrophages. (×1620.)

Fig. 4.—Adeno-carcinoma 27 (122 C) in initial phase of absorption. (×1000.)

Fig. 5.—Adeno-carcinoma 27 (136 C). Group of surviving cells when the tumour was nearing complete absorption. (×1620.)

Fig. 6.—Alveolar carcinoma 91 (153 B). Internal apparatus in different stages of fragmentation during spontaneous absorption. (×1620.)

Fig. 7.—Alveolar carcinoma 206 (331 A). Breaking up of reticular apparatus during absorption. (×1620.)

Fig. 8.—From connective-tissue capsule enveloping keratinized remains of squamous cell carcinoma 630 (126 A). Connective-tissue cells and foreign body giant cells with fragmented apparatus. (×800.)

Fig. 9.—From the peripheral layers of an absorbing nodule of sarcoma 37 S (140 A). (×1240.)